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Appl. No10/681,753
Amdt. dated March 24, 2010
Reply to Office action of November 24, 2009

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1 to 52. (Canceled)

53. (Currently Amended) A method of producing a multi-layered tissue construct *in vivo* comprising the steps of:

providing a first polymerizable mixture which comprises a first polymerizable material and first cells and directly placing a volume of the first polymerizable mixture in an osteochondral tissue space wherein the first polymerizable mixture is in direct contact with the osteochondral tissue underneath, then crosslinking the first polymerizable mixture material until the first polymerizable mixture material is at least partially gelled to form a first layer of hydrogel;

providing a second polymerizable mixture which comprises a second polymerizable material and directly placing a volume of the second polymerizable mixture in the tissue space with the at least partially gelled first layer of hydrogel, then crosslinking the second polymerizable mixture material until the second polymerizable mixture material is at least partially gelled to form a second layer of hydrogel; and

~~placing a volume of the first polymerizable mixture in an osteochondral tissue space wherein the first polymerizable mixture is in direct contact with the osteochondral tissue underneath, then crosslinking the first polymerizable mixture material until the first polymerizable mixture material forms an at least is partially gelled to form a first layer of hydrogel;~~

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~~placing a volume of the second polymerizable mixture in the tissue space with the at least partially gelled first layer of hydrogel, then crosslinking the second polymerizable mixture material until the second polymerizable mixture material is at least partially gelled to form a second layer of hydrogel; and~~

additionally crosslinking the first and second polymerizable mixtures materials until the first and second layers further polymerize to form an integrated multi-layered gel hydrogel;

wherein the first and second hydrogels each independently consist essentially of a hydrophilic and synthetic polymer network with high water content.

54. (Previously Presented) The method according to claim 53, wherein the first polymerizable mixture further comprises a polymerization initiator.

55. (Previously Presented) The method according to claim 53, wherein the second polymerizable mixture further comprises a polymerization initiator.

56. (Previously Presented) The method according to claim 53, wherein the second polymerizable mixture further comprises second cells.

57. (Previously Presented) The method according to claim 56, wherein the first and second cells are different cell types.

58. (Previously Presented) The method according to claim 57, wherein the first cells are mesenchymal stem cells and the second cells are chondrocytes.

59. (Previously Presented) The method according to claim 56, wherein the first and second cells are same cell types.

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60. (Previously Presented) The method according to claim 59, wherein the first and second cells are chondrocytes.

61. (Previously Presented) The method according to claim 53, wherein the cells are autologous cells.

62. (Previously Presented) The method according to claim 53, further comprising the step of adding a suspension of cells to a surface of the at least partially gelled first layer, before the step of placing the volume of the second polymerizable mixture in the tissue space.

63. (Previously Presented) The method according to claim 53, wherein at least one of the first and second polymerizable mixtures further comprises a bioactive substance selected from the group consisting of a nutrient, a cellular mediator, a growth factor, a compound which induces cellular differentiation, a bioactive polymer, a gene vector, and a pharmaceutical.

64. (Canceled)

65. (Previously Presented) The method according to claim 53, wherein crosslinking of the first polymerizable mixture material is controlled by exposure to an external radiation, and crosslinking of the second polymerizable mixture material is controlled by exposure to the external radiation.

66. (Currently Amended) A method of producing a multi-layered tissue construct *in vivo* comprising the steps of:

providing a first polymerizable mixture which comprises a first polymerizable material and first cells and directly placing a volume of the first

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polymerizable mixture in an osteochondral tissue space wherein the first polymerizable mixture is in direct contact with the osteochondral tissue underneath, then crosslinking the first polymerizable mixture material until the first polymerizable mixture material forms an at least is partially gelled to form a first layer of hydrogel;

providing a second polymerizable mixture which comprises a second polymerizable material and directly placing a volume of the second polymerizable mixture in the tissue space with the at least partially gelled first layer, then crosslinking the second polymerizable mixture material until the second polymerizable mixture material is at least partially gelled to form a second layer of hydrogel;

providing a third polymerizable mixture which comprises a third polymerizable material and directly placing a volume of the third polymerizable mixture in the tissue space with the at least partially gelled first and second layers, then crosslinking the third polymerizable mixture material until the third polymerizable mixture material is at least partially gelled to form a third layer;

wherein at least one of the second and third polymerizable mixtures further comprises cells;

~~placing a volume of the first polymerizable mixture in an osteochondral tissue space wherein the first polymerizable mixture is in direct contact with the osteochondral tissue underneath, then crosslinking the first polymerizable mixture material until the first polymerizable mixture material forms an at least is partially gelled to form a first layer of hydrogel;~~

~~placing a volume of the second polymerizable mixture in the tissue space with the at least partially gelled first layer, then crosslinking the second polymerizable mixture material until the second polymerizable mixture material is at least partially gelled to form a second layer of hydrogel;~~

~~placing a volume of the third polymerizable mixture in the tissue space with the at least partially gelled first and second layers, then crosslinking the~~

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~~third polymerizable mixture material until the third polymerizable mixture material is at least partially gelled to form a third layer; and~~

additionally crosslinking the first, second, and third polymerizable mixtures materials until the first, second, and third layers further polymerize to form an integrated multi-layered gel hydrogel; and

wherein the first, second, and third hydrogels each independently consist essentially of a hydrophilic and synthetic polymer network with high water content.

67. (Previously Presented) The method according to claim 66, wherein the first polymerizable mixture further comprises a polymerization initiator.

68. (Previously Presented) The method according to claim 66, wherein the second polymerizable mixture further comprises a polymerization initiator.

69. (Previously Presented) The method according to claim 66, wherein the third polymerizable mixture further comprises a polymerization initiator.

70. (Previously Presented) The method according to claim 66, wherein the second polymerizable mixture further comprises second cells, and the third polymerizable mixture further comprises third cells.

71. (Previously Presented) The method according to claim 70, wherein the first, second, and third cells are different cell types.

72. (Previously Presented) The method according to claim 71, wherein the first cells are mesenchymal stem cells and the second cells are chondrocytes.

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73. (Previously Presented) The method according to claim 70, wherein the first, second, and third cells are same cell types.

74. (Previously Presented) The method according to claim 73, wherein the first, second, and third cells are chondrocytes.

75. (Previously Presented) The method according to claim 66, wherein the cells are autologous cells.

76. (Previously Presented) The method according to claim 66, further comprising the step of adding a suspension of cells to a surface of the at least partially gelled first layer, before the step of placing the volume of the second polymerizable mixture in the tissue space.

77. (Previously Presented) The method according to claim 66, wherein at least one of the first, second, and third polymerizable mixtures further comprises a bioactive substance selected from the group consisting of a nutrient, a cellular mediator, a growth factor, a compound which induces cellular differentiation, a bioactive polymer, a gene vector, and a pharmaceutical.

78 to 87. (Canceled)